

## AMENDMENTS TO THE CLAIMS

This listing of the claims will replace all prior versions, and listings, of claims in the application:

### Listing of the Claims

1. (currently amended) A method of preparing a microanalytical device, for immobilizing a biological molecule in a porous inorganic matrix, said method comprising forming a sol-gel comprising an entrapped biological molecule; crushing the sol-gel into particulates having a diameter of from about 10  $\mu$ m to about 80  $\mu$ m, to form sol-gel particulates; and, forming the sol-gel particulates into a bed within a microanalytical device or on the surface of the microanalytical device;  
~~the forming an aqueous composition comprising a ceramic oxide colloidal sol mixed with an acidified oxide salt solution;~~  
~~adding to said composition an amount of the biological molecule in a physiologically acceptable buffered solution, said aqueous composition becoming turbid on being transformed into a polymerizing hydroxide solution and transforming to a gel;~~  
~~shaping the gel produced in step (b) into a final form;~~  
~~aging the gel; and~~  
~~crushing the aged gel into particulates, wherein the crushed gel particulates are between about 10  $\mu$ m and about 80  $\mu$ m in diameter and are suitable for incorporation into a microanalytical device;~~  
wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained.
2. (currently amended) The method of claim 1, wherein after the sol-gel is formed and before the sol-gel is crushed, the sol-gel is aged for about two weeks.
3. (currently amended) The method of claim 1, wherein after the sol-gel is formed and before the sol-gel is crushed, the sol-gel is aged at a temperature of from about 4°C to about 40°C.

Claim 4 – 8 (canceled)

9. (currently amended) A method for immobilizing a biological molecule in a porous inorganic sol-gel matrix incorporated into a microanalytical device, said method comprising

forming a sol-gel ~~an aqueous~~ composition comprising a tetraalkyl orthosilicate and a silane, wherein the silane is substituted with a C<sub>8</sub>-C<sub>24</sub> alkyl group and substituted with at least two leaving groups selected from OR and halo, ~~mixed with an acidified oxide salt solution;~~

adding to said composition an amount of the biological material to entrap one or more biological molecules into the sol-gel ~~in a physiologically acceptable buffered solution wherein the resulting aqueous composition has a pH ranging from about 6 to about 8.5, said aqueous composition becoming turbid on being transformed into a polymerizing hydroxide solution and transforming to a gel;~~

crushing the sol-gel into particulates having a diameter of from about 10  $\mu$ m to about 80  $\mu$ m; and,

forming the sol-gel particulates into a bed within a microanalytical device or on the surface of the microanalytical device;

~~shaping the gel produced in step (b) into a final form; and~~

~~aging the gel;~~

wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained; and wherein the porous inorganic sol-gel matrix is formed in situ.

Claims 10-14 (canceled)

15. (previously presented) The method of claim 9, wherein the tetraalkyl orthosilicate is selected from the group consisting of tetra-ethyl orthosilicate, tetra-methyl orthosilicate, and combinations thereof.

16. (original) The method of claim 1, wherein the sol is comprised of colloidal silica sol and a dissolved metal silicate.

17. (original) The method of claim 16, wherein the metal silicate is sodium silicate.

18. (original) The method of claim 1, wherein the sol comprises a tetraalkyl orthosilicate and a silane substituted with at least two leaving groups selected from the group consisting of OR and halo.

19. (original) The method of claim 18, wherein the silane is substituted with a C<sub>8</sub>-C<sub>24</sub> alkyl group.

20. (original) The method of claim 19, wherein the alkyl group is C<sub>18</sub>.

21. (previously presented) The method of claim 18, wherein the tetraalkyl orthosilicate is selected from the group consisting of tetra-ethyl orthosilicate, tetra-methyl orthosilicate, and combinations thereof.

Claims 22-23 (canceled)

24. (currently amended) The method of claim 2, wherein the particle size of the ~~ceramic oxide colloidal~~ sol-gel is selected to produce pores when the sol-gel is aged, said pores being of a diameter which is approximately the same as the diameter of the biological molecule to be entrapped.

Claim 25 (canceled)

26. (original) The method of claim 24, wherein the pores have an average diameter ranging from about 1 nm to about 100 nm.

27. (currently amended) The method of claim ~~{1}~~ 26, wherein the pores have an average diameter ranging from about 2 nm to about 50 nm.

28. (previously presented) The method of claim 1, wherein the pores have a diameter which is approximately the same as the diameter of the biological molecule to be entrapped.

29. (original) The method of claim 28, wherein the diameter of the pores is less than the diameter of the entrapped biomolecule.

30. (currently amended) The method of claim 9, wherein the gel produced prior to crushing in step (b) is shaped into forms selected from the group consisting of a monolithic gel, thin film, or fiber.

31. (original) The method of claim 9, wherein the pores have an average diameter ranging from about 1 nm to about 100 nm.

32. (original) The method of claim 31, wherein the pores have an average diameter ranging from about 2 nm to about 50 nm.

33. (original) The method of claim 24, wherein molecules having a mass of 3,000 Da or less can diffuse through the pores.

34. (original) The method of claim 24, wherein molecules having a mass of 5,000 Da or less can diffuse through the pores.

35. (original) The method of claim 24, wherein molecules having a mass of 10,000 Da or less can diffuse through the pores.

36. (original) The method of claim 24, wherein molecules having a mass of 15,000 Da or less can diffuse through the pores.

37. (original) The method of claim 28, wherein molecules having a mass of 3,000 Da or less can diffuse through the pores.

38. (original) The method of claim 28, wherein molecules having a mass of 5,000 Da or less can diffuse through the pores.

39. (original) The method of claim 28, wherein molecules having a mass of 10,000 Da or less can diffuse through the pores.

40. (original) The method of claim 28, wherein molecules having a mass of 15,000 Da or less can diffuse through the pores.

41. (original) The method of claim 1, wherein the biological molecule is selected from the group consisting of polynucleotides, enzymes, antibodies, coagulation modulators, cytokines, endorphins, peptidyl hormones, kinins, receptors, genes, gene fragments, cell fragments, membrane fragments, and solubilized membrane proteins.

42. (previously presented) The method of claim 41, wherein the enzyme is selected from the group consisting of RNase, DNase, telomerase, ligase, nuclease, ribonuclease; hydrogenase, dehydrogenase, aldase, amidase, aminotransferase, amylase, anhydrase, apyrase, arginase, aspartase, aspariginase, carboxylase, carboxypeptidase, catalase, cellulase, cholinesterase, acetylcholinesterase, deaminase, dextranase, dismutase, elastase, esterase, fumarase, glucosidase, hexokinase, isomerase, invertase, kinase, lactase, lipase, lysozyme, malase, naringinase, oxidase, oxygenase, papain, pectinase, peptidase, pepsin, peroxidase, phosphodiesterase, phosphotase, protease, reductase, transferase, tyrosinase, urase, trypsin, chymotrypsin, hydrolases, isomerases, proteases, ligases and oxidoreductases such as esterases, phosphatases, glycosidases and peptidases, superoxide dismutase, tissue plasminogen activator, renin, adenosine deaminase, alpha-glucocerebrosidase, aspariginase, dornase-alpha, hyaluronidase, elastase, trypsin, thymidine kinase, tryptophan hydroxylase, urokinase, kallikrein, bromelain, cathepsins B, D, G, C, clostripain, endoproteinase Arg C, endoproteinase Asp N, endoproteinase Glu C, endoproteinase Lys C, Factor Xa, proteinase K, subtilisin, thermolysin, acyloamino acid releasing enzyme, aminopeptidases, carboxypeptidases, and pyroglutamate aminopeptidase.

43. (currently amended) The method of claim 1, wherein the average sol-gel particle size is from about 1 nm to about 30 nm.

44. (original) A method of preparing a microanalytical device, comprising forming a sol-gel comprising an entrapped biological molecule, crushing the sol-gel to particulates having

a diameter of from about 10  $\mu\text{m}$  to about 80  $\mu\text{m}$ , and forming the sol-gel particulates into a bed within the microanalytical device or on the surface of the microanalytical device.

45. (currently amended) ~~The A-method of claim 44, of preparing a microanalytical device comprising forming a sol-gel comprising an entrapped biological molecule,~~ wherein the form of said sol-gel is selected from the group consisting of a monolithic gel, thin film, or fiber and wherein the sol-gel is placed in or on the microanalytical device.

46. (currently amended) A method of using a microanalytical device prepared according to the method of claim 44 ~~comprising a sol-gel comprising an entrapped biological molecule, comprising forming the sol-gel into a bed within the microanalytical device or on the surface of the microanalytical device,~~ applying an analyte sample to the bed, optionally applying additional buffer solution to the bed, and analyzing the eluant from the bed.

47. (previously presented) The method of claim 44 or 46, wherein the bed on the microanalytical device is in the form of a microcolumn or microchannel.

48. (previously presented) The method of claim 44 or 46, wherein the bed on the microanalytical device is in the form of a microarray.

49. (original) The method of claim 46, wherein the eluant is analyzed using mass spectrometry.

50. (original) The method of claim 46, wherein the eluant is analyzed using micro or capillary electrophoresis.

51. (previously presented) The method of claim 46, wherein the interaction of any component in the sample with the entrapped biological molecule in the sol-gel is measured using a method selected from the group consisting of UV/Visible, Near IR, fluorescence, refractive index and Raman spectroscopies.



52. (original) The method of claim 46, further comprising washing the sol-gel with a solution to elute analytes from the sol-gel, and analyzing the analytes.

53. (original) The method of claim 52, wherein the analytes are analyzed using mass spectrometry.

54. (previously presented) The method of claim 52, wherein the analytes are analyzed using a method selected from the group consisting of UV/Visible, Near IR, fluorescence, refractive index and Raman spectroscopies.

55. (original) The method of claims 44 or 45, wherein the microanalytical device is fabricated by a method selected from the group consisting of silicon micromachining, microlithography, molding and etching.

56. (original) The method of claim 45, wherein the sol-gel is formed in situ on the microanalytical device.

Claim 57 (canceled)

58. (currently amended) In a microanalytical device comprised of a substrate and at least one feature selected from microchannels, microcolumns, and combinations thereof, the improvement which comprises incorporating into said at least one feature and/or onto a surface of the substrate a sol-gel having a biological molecule entrapped therein, wherein the sol-gel has been crushed into particulates having a diameter of from about 10  $\mu$ m to about 80  $\mu$ m and the particulates have been formed into a bed within the microanalytical device or on the surface of the microanalytical device ~~is in a form selected from the group consisting of a monolithic gel, a thin film, and a fiber.~~

59. (previously presented) The microanalytical device of claim 58, adapted for performing high throughput screening of samples.